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Comparative infectivity of oocysts and bradyzoites of *Toxoplasma gondii* for intermediate (mice) and definitive (cats) hosts

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Abstract

Tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts) are the three infectious stages of *Toxoplasma gondii*. The prepatent period (time to shedding of oocysts after primary infection) varies with the stage of *T. gondii* ingested by the cat. The prepatent period (pp) after ingesting bradyzoites is short (3–10 days) while it is long (18 days or longer) after ingesting oocysts or tachyzoites. The conversion of bradyzoites to tachyzoites and tachyzoites to bradyzoites is biologically important in the life cycle of *T. gondii* and it has been proposed that the pp can be used to study stage conversion. In the present study, infectivity of oocysts and bradyzoites released from tissue cysts of a recent isolate of *T. gondii*, TgCkAr23, to cats and mice was compared. Ten-fold dilutions of oocysts or bradyzoites were administered orally to cats, and orally and subcutaneously to mice. Of the 29 cats each fed 1–10 million oocysts only one cat shed oocysts and the pp was 23 days; all cats remained asymptomatic. In contrast, all mice administered the same 10-fold dilutions of oocysts either orally or subcutaneously died of toxoplasmosis. The results confirm that infectivity of the oocysts to cats is lower than for mice and that oocysts are non-pathogenic for cats. Of the 41 cats each fed 1–1000 free bradyzoites, 15 shed oocysts with a short pp of 4–9 days, and all remained asymptomatic. The infectivity of bradyzoites to mice by the oral route was approximately 100 times lower than that by the subcutaneous route. The results confirm the hypothesis that the pp in cats is stage and not dose dependent, and that transmission of *T. gondii* is most efficient when cats consume tissue cysts (carnivory) or when intermediate hosts consume oocysts (fecal-oral transmission).

Keywords: *Toxoplasma gondii*; Bradyzoites; Oocysts; Cats; Prepatent periods

1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked

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meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. Cats shed oocysts after ingesting any of the three infectious stages of *T. gondii*, i.e. tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts). Prepatent periods (time to the shedding of oocysts after initial infection) and frequency of oocyst shedding vary according to the stage of *T. gondii* ingested. Prepatent periods are 3–10 days after ingesting tissue cysts, 18 days or more after ingesting oocysts (Dubey, 1996, 2001, 2005; Dubey and Frenkel, 1972, 1976). The prepatent period after ingesting transitional stages between tachyzoites and bradyzoites may vary from 11 to 17 days (Dubey, 2002, 2005). *T. gondii* has also adapted to an oocyst-oral route in herbivores (intermediate hosts) and tissue cyst-oral route in carnivores, especially in the cat. *T. gondii* oocysts are less infective and less pathogenic for the cat than for other hosts (Dubey, 1996). For example, one live oocyst is orally infective to mice and pigs (Dubey et al., 1996) whereas 100 or more oocysts may be required to establish infection in a cat (Dubey, 1996). The reverse may be true for bradyzoites. By mouth, bradyzoites are less infective to mice than cats (Dubey, 2001). Cats can shed millions of oocysts after ingesting as few as one bradyzoite whereas 100 bradyzoites may not be infective to mice by the oral route (Dubey, 2001). However, these quantitative data were obtained mainly using the VEG (Type III) and the Me 49 (Type II) strains of *T. gondii* that had been maintained in the laboratory for more than a decade and had undergone an unknown number of passages in mice (Dubey et al., 1996; Dubey, 1997)

Recently, it has been proposed that the transmission and pathogenicity of *T. gondii* are genetically dependent. There are at least three genetic types of *T. gondii* (Howe and Sibley, 1995). Type I strains are considered more pathogenic than Type II and III strains, but this information is based on experiments in mice (Howe and Sibley, 1995; Howe et al., 1996, 1997). There is circumstantial evidence that Type I strains are associated with clinical toxoplasmosis in humans with ocular disease and in patients with acquired immunodeficiency syndrome (Boothroyd and Grigg, 2002; Khan et al., 2005).

The objective of the present investigation was to quantitatively compare infectivity of oocysts and bradyzoites of a recent Type I isolate of *T. gondii* (TgCkAr23) both in the definitive host, the cat, and in an intermediate host, the mouse.

2. Materials and methods

2.1. Isolate of T. gondii

The TgCkAr23 was isolated from an asymptomatic chicken no.73 from Argentina in September 2004 (Dubey et al., 2005). It is a Type I strain and is pathogenic for Swiss Webster (SW) mice (Dubey et al., 2005). The original passage of the strain was used in this study. Five SW mice were inoculated subcutaneously (s.c.) with brain homogenate of this chicken. All five mice became sick and died or were euthanized on days 19, 19 or, 35 post-inoculation (p.i.). Tachyzoites were seen in the lungs of mice at day 19 p.i. and tissue cysts were seen in the brains of mice at 35 days p.i.; these tissue cysts were used to obtain oocysts in the present study.

To obtain tissue cysts, SW mice were inoculated subcutaneously (s.c.) with oocyst-derived tachyzoites (Dubey et al., 2005) and treated with sulfadiazine sodium (1 mg per ml of drinking water). The mice were killed 70 days p.i. and their brains were homogenized with a pestle and mortar in a 0.9% NaCl solution (saline). Tissue cysts were separated from brain in a Percoll gradient (Cornelissen et al., 1981), washed, and bradyzoites were released by acid pepsin treatment (Popiel et al., 1996). After washing, the bradyzoite suspension was passed through a 3-μm membrane filter (Nuclepore, Pleasanton, CA) and bradyzoites were counted in a hemocytometer.

2.2. Infectivity of T. gondii in cats

Seventy *T. gondii*-free cats from a closed colony were used. The management of cats, method of feces collection, and oocyst enumeration were described in detail (Dubey, 1995, 2005). All experiments were performed as per the guidelines of the U.S. Department of Agriculture Animal Care Committee. For bioassay, suspensions of *T. gondii* were poured into the mouth by holding of the cat head in an upward position. Antibodies to *T. gondii* were determined in sera of cats by using the modified agglutination test

(MAT) as described (Dubey and Desmonts, 1987); antibodies to *T. gondii* were not found in 1:25 dilution of serum of any of the 70 cats prior to use in this study.

Feces of each cat were examined microscopically for oocysts. For this, the entire sample was emulsified with a small volume of water, and then ~10 g were mixed with ~40 ml of a sucrose solution (specific gravity, 1.18), filtered through gauze, and centrifuged in a 50-ml tube at 2000 rpm (~1200 g) for 10 min. A drop of the float from the meniscus was examined microscopically for oocysts. If oocysts were detected, the entire daily sample was mixed with sucrose solution in 50-ml tubes and centrifuged at 2000 rpm for 10 min. Then supernatant in the 50 ml tube was mixed with 200 ml of water and centrifuged. The supernatant was discarded, the sediment was mixed with water, and all samples from each cat for each day were pooled, centrifuged, and finally suspended in water to make a final volume of 100 ml. Fecal floats were incubated in 2% sulfuric acid for 1 week at room temperature on a shaker to allow sporulation of oocysts and then stored at 4 °C.

2.3. Experiment 1

Sporulated oocysts that had been stored for 9 months in 2% sulfuric acid at 4 °C were washed free of acid and suspended in saline. Ten-fold dilutions were made in saline starting with 10 million oocysts per ml. The first dilution contained 1 million oocysts per ml and the last dilution was estimated to have few or no oocysts (Table 1). Aliquots (1 ml for each cat and 0.5 ml for each mouse) from each oocyst dilution were administered to 29 cats orally and to 80 mice orally and by the s.c. routes (Table 1). Feces of cats were observed for oocyst shedding for 4 weeks. At the termination of the experiment the cats were euthanized and a blood sample obtained for serology.

Outbred female SW mice obtained from Taconic Farms, Germantown, New York were used for bioassay. Tissue imprints of lungs and brains of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 41 post-inoculation (p.i.) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 47 or 48 days p.i. and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

2.4. Infectivity of bradyzoites to cats and mice

Ten-fold dilutions of free bradyzoites were made in saline and aliquots (1 ml for each cat and 0.5 ml for each mouse) were administered orally to cats and to mice orally and s.c. (Tables 2 and 3). The major objective was to determine prepatent period in cats fed a few viable bradyzoites. Two trials were performed. In trial 1, bradyzoite dilutions were fed to 21 cats and inoculated s.c. and orally into 50 mice (Table 2). In trial 2, bradyzoite suspensions were fed to 20 cats and inoculated orally and s.c. into 50 mice (Table 3). Feces of cats were examined for oocysts shedding for 21

Table 1
Comparative infectivity of *T. gondii* oocysts to cats and mice

Cats				Mice[a]	
No. of oocysts administrated	No. fed	No. shed oocysts	No. seroconverted[b]	No. infected/five inoculated	
				Oral	s.c.
1000000	12	0	12	5	5
100000	1	0	1	5	5
10000	4	0	3	5	5
1000	4	1	3	5	5
100	4	0	1	5	5
10	4	0	0	4	5
1	Not fed			2	2
<1	Not fed			1	1

[a] All *T. gondii* infected mice died of toxoplasmosis.
[b] MAT antibodies of 1:25 in one, and 1:200 or higher in 19 cats, 4 weeks p.i.

Table 2
Comparative infectivity of free *T. gondii* bradyzoites to cats and mice

Dosage	No. of cats fed bradyzoites	No. shed oocysts	Infectivity of bradyzoites to mice/five mice inoculated	
			Oral	s.c.
1000	3	1	3	5
100	6	2	0	4
10	6	1	0	3
1	6	1	0	2
<1	Not fed	Not applicable	0	0

Table 3
Comparative infectivity of free *T. gondii* bradyzoites to cats and mice

Dose	No. cats fed bradyzoites	No. shed oocysts	Infectivity of bradyzoites to mice/five mice inoculated	
			Oral	s.c.
1000	4	2	5	5
100	4	4	4	5
10	4	3	3	5
1	4	1	0	3
<1	4	0	0	0

days. The mice were observed for 7 weeks and examined as described above.

3. Results

3.1. Experiment 1

All cats fed oocysts remained asymptomatic during an observation period of 4 weeks. Only 1 of the 29 cats fed 1–10 million oocysts shed oocysts; the prepatent period was 23 days and the cat had been fed 1000 oocysts. Twenty of the 29 cats became seropositive; 19 had MAT titers of 1:200 or higher and one had a titer of 1:25, 4 weeks after feeding oocysts (Table 1).

3.2. Experiment 2

Infectivity of free bradyzoites to cats differed in two trials. In trial 1, only five of the 21 cats fed bradyzoites shed oocysts (Table 2). In trial 2, 10 of 16 cats fed bradyzoites shed oocysts (Table 3). The prepatent period in all cats was short (4–9) days, irrespective of the dose.

The infectivity of the free bradyzoites to mice varied by the route, bradyzoites were 100-fold more infective by the subcutaneous versus the oral route (Tables 2 and 3) and infected mice either died or were euthanized because they were ill. In trial 1, 14 of 20 (70%) mice inoculated s.c. became infected versus only 3 of 20 (15%) mice inoculated by the oral route. In trial 2, 8 of 20 (90%) mice were infected by the s.c. route versus the 12 of 20 (60%) mice by the oral route.

4. Discussion

Results of the present study using a Type I *T. gondii* strain confirmed our earlier results obtained with a Type III strain (Dubey, 1996) that oocysts are not pathogenic and less infective for cats than for mice. Seroconversion data suggested that infectivity of the oocysts in cats was dose dependent; all 13 cats given 100 000 or 1 000 000 oocysts seroconverted, six of eight cats fed 1000 or 10 000 oocysts seroconverted, only one of four given 100 oocysts seroconverted, whereas none of the four cats given 100 oocysts seroconverted within 4 weeks p.i. (Table 1). Mouse

infectivity data indicated that the oocysts given to cats were viable because 6 of 20 mice from the inocula estimated to have only one oocyst became infected. All cats fed oocysts remained asymptomatic whereas all infected mice died of toxoplasmosis, irrespective of the dose. It is noteworthy that infectivity of the oocysts for mice by the subcutaneous route was similar to that by the oral route.

The infectivity of the bradyzoites for cats and mice was different than oocysts. In trial 1, only 5 of the 21 cats fed bradyzoites shed oocysts although the same inocula were infective for mice by the s.c. route (Table 2). In trial 2, 10 of the 16 cats fed free bradyzoites shed oocysts (Table 3). The poor infectivity of bradyzoites for cats in the first trial is unexplained. The inocula containing free bradyzoites were poured in the mouth of the cat and some bradyzoites might have been destroyed before reaching the small intestine to initiate the schizogonic cycle (Dubey and Frenkel, 1972). In the case of mice the inocula were deposited directly in the stomach by a feeding needle.

In the present study cats were fed low numbers of free bradyzoites because I wanted to determine prepatent period after cats had been fed one or a few bradyzoites. It is noteworthy that 2 of 10 cats fed an estimated one bradyzoite shed oocysts. The prepatent period for oocysts shedding by cats in both trials was 4–9 days. The results confirm the hypothesis that prepatent periods are stage and not dose dependent. Until recently it was believed that tachyzoites of *T. gondii* are not infectious orally and that a reliable method to obtain tachyzoites was from the peritoneal exudates (pex) of mice inoculated with *T. gondii* because bradyzoites were not present in the pex. In a recent study, 21 of 38 cats fed pex from the mice inoculated with the TgCkAr 23 isolate (used in the present study) shed oocysts with a short prepatent (pp 5–10 days) in 10 cats, intermediate (pp 11–17 days) in six cats, and long pp in five cats (Dubey, 2005). These results were interpreted to suggest that short pp was due to bradyzoites in the inoculum, intermediate pp was due to transitional stages between tachyzoites and bradyzoites, and long pp was related to the ingestion of tachyzoites. Results of the present study using a high dose of oocysts and a low number of bradyzoites of the TgCkAr23 isolate support this hypothesis that the pp is stage and not dose dependent.

Results of the present study confirm that cats can shed oocysts after ingesting even a few bradyzoites of the Type I strain and that bradyzoites are less infective for mice by the oral route (Dubey, 2001). The present study was conducted using a low passage strain of *T. gondii* because prolonged passage of *T. gondii* in mice or cell culture can alter the biologic behavior of a strain. For example, the RH strain of *T. gondii* may not produce tissue cysts in mice, it may not persist in inoculated pigs and rats, and its tissue may not be infective for cats and mice (Dubey et al., 1999; Frenkel et al., 1970, 1976; Villard et al., 1997).

In the present study, free bradyzoites and not intact tissue cysts were used to quantitatively compare infectivity in cats and mice. The number of bradyzoites in a tissue cyst at any stage of infection in mice can vary more than 1000-fold, some tissue cysts may have only a few whereas others may have several thousand bradyzoites (Dubey et al., 1998). To my knowledge, quantitative comparisons of the oral and parenteral infectivity of tissue cysts or free bradyzoites for mice has been reported only for seven strains of *T. gondii* (Table 4). In each case the

Table 4
Mouse infectivity of the tissue cysts or bradyzoites of different strains of *T. gondii*

Strain (genotype)	Inoculum	Infective dose		Reference
		Oral	i.p. or s.c.	
Human fetus	Bradyzoites	100	i.p. 1[a]	Dubey et al. (1981)
Cat 5	Bradyzoites	100	i.p. 1	Dubey et al. (1981)
GT 1 (Type I)	Tissue cysts	100	i.p. 1	Dubey (1980)
M-7741	Tissue cysts	100	i.p. 1	Dubey and Frenkel (1973)
VEG (Type III)	Bradyzoites	1000	s.c. 1	Dubey (1997)
Me 49 (Type II)	Bradyzoites	100	s.c. 1	Dubey (1997)
TgCkAr23 (Type I)	Bradyzoites	100	s.c. 1	Present study

[a] Based on infectivity in mice.

infectivity was 100-fold lower by the oral route compared with the parenteral route. For experiments, tissue cysts are normally obtained from the brain of infected mice and they are freed from the neural tissue by homogenizing brain tissue in saline. During this homogenizing procedures a few tissue cysts are ruptured mechanically and released bradyzoites are not accounted for during enumeration of tissue cysts. Therefore, tissue cysts were purified by Percoll and bradyzoites were freed by enzymatic digestion of the tissue cyst wall.

In conclusion, results support the hypothesis that *T. gondii* is biologically adapted to transmission by carnivory in cats and by fecal-oral route in herbivores. Humans can become infected both by ingestion of oocysts and infected meat but unfortunately there are no tests at the present time to determine the efficiency of meat versus oocyst transmission. Why *T. gondii* infections are more serious clinically in some patients is speculative and there are no data at the present time to indicate that the parasite genotype is a determining factors in higher mammals other than mice. Results of the present study suggests that the stage of the parasite ingested (bradyzoite versus oocysts) and the host (cat versus mouse) are a factor in determining pathogenicity of *T. gondii*.

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